Multi-drug Resistant Pseudomonas aeruginosa and Acinetobacter baumannii Infections among Hospitalized Patients: Risk Factors and Outcomes

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Abstract
Aims and Objectives: Pseudomonas aeruginosa [PA] and Acinetobacter baumannii [AB] are important nosocomial pathogens in healthcare settings. Treatment is complicated by multi-drug resistance [MDR]. Increasing resistance to carbapenems mediated by metallobetalactamase [MBL] and other mechanisms is a cause for concern because they adversely affect clinical outcomes and add to treatment costs. This study was undertaken to determine the prevalence of MBL production in carbapenem-resistant isolates and to study the factors influencing the clinical outcomes of infections.

Methods: Fifty-five carbapenem-resistant nosocomial isolates of PA (30) and AB (25) were included for the study. Multidrug resistance was defined as being resistant to all classes of antibiotics including carbapenems. This was determined by disc diffusion method in accordance with CLSI. Minimum inhibitory concentration [MIC] to imipenem and meropenem was done by agar dilution method. MBL production was detected using ethylene diamine tetraacetic acid [EDTA] as inhibitor both by disc diffusion and MIC testing. Risk factors related to hospital and ICU stay were analysed. Outcomes were followed up. Proportions were compared using Chi square test to determine the factors influencing the outcome. Differences were considered significant if P was <0.05.

Results: All isolates exhibited moderate to high degree of resistance to carbapenems. Their MIC ranged from 8-2048 mcg/ml. Crossresistance to cephalosporins, fluoroquinolones, aminoglycosides and beta lactam-betalactamase combination was seen in all isolates. Ninety two percent were susceptible to polymyxins.

MBL was detected in 44 isolates [PA-29, AB-15], while 11 isolates were negative. The common site of isolation was the respiratory tract (41.8%) followed by urinary tract (25.5%), wound (20%) and blood (12.7%). Colonisation from infection was delineated based on clinical and laboratory criteria. Death occurred in 57% of patients. Factors contributing to mortality were length of hospital and ICU stay (P=0.001), intubation (P=0.0005), usage of multiple invasive devices and presence of a focal or a generalized infection (P=0.001). Administration of multiple antibiotics did not affect the mortality.

Conclusions: MBL-mediated carbapenem resistance in PA and AB is a significant threat in hospitalised patients. It should be addressed with infection control measures, surveillance and alternative new therapeutic strategies.

Introduction
Antibiotic resistance is a major concern of contemporary medicine. The continuing emergence of resistant strains that cause nosocomial infections contributes substantially to the morbidity and mortality among hospitalized patients. Of the nosocomial pathogens, Pseudomonas aeruginosa and Acinetobacter baumannii are of greatest concern for hospitalized patients particularly those in intensive care units [ICU] where these pathogens are capable of causing severe invasive infections in critically ill and immunocompromised patients. Antimicrobial resistance among nosocomial isolates of Pseudomonas aeruginosa and Acinetobacter baumannii, complicates the treatment of infections and adversely affects clinical outcomes and patient treatment costs.1,2

The advent of carbapenems in the 1980s heralded a new treatment option for serious bacterial infections. However, resistance to carbapenems has been frequently observed in Gram-negative bacilli such as Pseudomonas aeruginosa and Acinetobacter baumannii. The common form of resistance is mediated by lack of drug penetration (i.e., porin mutations and efflux pumps) and/or carbapenem hydrolysing betalactamase enzymes including the metallobetalactamases (MBL). MBLs are enzymes requiring divalent cations, usually zinc as metal cofactors for enzyme activity, being inhibited by the action of metal ion chelator such as ethylene diamine tetraacetic acid (EDTA). The MBLs efficiently hydrolyse all beta-lactams, except aztreonam in vitro.3,4 Acquired MBLs are encoded mobile gene cassettes of organism and such strains are often resistant to different groups of antimicrobial agents with transferable properties to various types of bacteria.5 The rapid detection of MBL producing Gram-negative bacilli is therefore necessary to aid in infection control measures and to prevent their dissemination.6

Because of the prevalence of multiple resistance, MBL producing isolates are often refractory to all the other treatment options, signaling the need for the development of new, potent therapeutic agents with novel modes of action. Many hospitals are forced to resort to older and more toxic drugs such as colistin
Materials and Methods

The study was conducted for a period of 5 months. It included 55 non-replicate, consecutive, clinically significant carbapenem-resistant Pseudomonas aeruginosa (n=30) and Acinetobacter baumannii (n=25) recovered from clinical specimens of patients hospitalized for >48 hours.

A multidrug resistant isolate was defined as being resistant to several classes of antibiotics including carbapenems by the disc diffusion method. The isolates were obtained from clinical specimens such as blood, pus, wound swabs, urine and lower respiratory secretions (bronchoalveolar lavage, bronchial wash and endotracheal secretions). Care was taken to differentiate commensals from pathogens for isolates obtained from nonsterile sites (respiratory tract, urinary tract and wound swabs). Significance of the isolates was based on the presence of the organism in the Gram stain, presence of intracellular forms of the organism and pure growth in culture with significant colony count. A correlative detailed clinical history was also sought.

Microbiological Procedures

Susceptibility to various classes of antibiotics was determined by disc diffusion method in accordance with Clinical Laboratory Standard Institute [CLSI] guidelines. The antibiotics tested were amikacin, piperacillin, ciprofloxacin, ceftazidime, piperacillin-tazobactam, aztreonam, imipenem, colistin and polymyxin B. MIC to imipenem and meropenem for these isolates was done by agar dilution method in accordance with CLSI standards.

Inhibition of the production of MBL enzymes was determined in the laboratory by three methods, zone enhancement with EDTA impregnated imipenem and ceftazidime discs, double disc synergy test (DDST) using imipenem and EDTA as inhibitor and minimum of four–fold reduction in MIC of the isolates with imipenem - EDTA combination.

Clinical Data

The patients’ clinical records were perused from the time of admission. The clinical data collected included the demographic characteristics, provisional diagnosis, duration of hospital stay, presence of indwelling devices including mechanical ventilators, period of stay in ICU, severity of illness (Acute Physiology and Chronic Health Evaluation–APACHE II score within the first 24 hours of admission in the ICU for adults patients), classes of antibiotics used, presence of focal or generalized infections, surgical intervention if any and presence of underlying diseases such as diabetes mellitus, chronic renal failure etc. The treatment given and the outcomes were followed up.

Statistical Analysis

Proportions were compared using Chi square test to determine the factors influencing the outcome of infections with carbapenem-resistant isolates. Differences were considered significant if P was <0.05. Analyses were performed using SPSS software.

Results

Antimicrobial Susceptibilities and MBL Screening

All the multidrug resistant isolates exhibited resistance to both imipenem and meropenem by the disc diffusion method. These isolates also had raised MIC values to imipenem and meropenem ranging from 8-2048 μg/ml [normal range- susceptible if MIC was <4 μg/ml, intermediate - 8 μg/ml and resistant - >16 μg/ml].

They were also resistant to fluoroquinolones, aminoglycosides and beta-lactam–beta-lactamase inhibitor combination drugs. A high proportion of isolates (92%) were susceptible to colistin and polymyxin B.

Majority of the isolates (n=44) exhibited a significant zone size enhancement, zone distortion and MIC reduction with EDTA, thereby suggesting production of MBL enzymes with potential for transfer of resistance. Only 11 isolates were susceptible to aztreonam which was tested by the disc diffusion method. Only a small proportion of the strains (n=11) with raised MIC to imipenem and meropenem did not exhibit changes with EDTA thereby suggesting other nontransferrable mechanisms of resistance.

Most of the isolates were obtained from the respiratory tract (41.8%) followed by urinary tract (25.5%), wound (20%) and blood (12.7%). Since only a minority of these isolates were from sterile body sites, emphasis was placed on a detailed clinical history to rule out colonization.

Description of Case Patients and Statistical Analysis

The mean age of the study patients was 41.4 years and the range was < 1 yr to 80 years. Among the 55 patients 40 (77%) were males and 15 (19%) were females. The average length of hospital stay was 24.4 days. Average duration of stay in ICU (n=48) was 20.6 days, of which 26 patients stayed for more than 14 days and 22 patients for less than 14 days. In the former group 16 expired and 10 survived while in the latter 15 expired and 7 survived.

A high mortality rate was observed in these patients (57%). The mean APACHE II score on admission for adult patients was 17.4±7.

Individual factors influencing mortality in patients infected with multidrug resistant organism is shown in Table 1. Variables found to be influencing the mortality in these patients were stay in ICU(P=0.001), intubation (P=0.0005) and usage of multiple invasive devices such as vascular and urinary catheters. Presence of a focal or a generalized infection (P=0.001) was a significant factor influencing the mortality in these patients.

The age and sex did not have any influence on the mortality in this study. Most of the patients received multiple antibiotics including carbapenem, but this factor did not affect the mortality.

Underlying illness such as diabetes mellitus, chronic renal failure and malignancy also had no direct impact on the mortality in the study patients.

Discussion

Resistance to antimicrobial agents is an increasing public health threat. It limits the therapeutic options and leads to increased mortality and morbidity. We conducted this study to understand the mechanisms of carbapenem resistance and also the individual risk factors influencing the mortality in these patients with infections caused by multidrug resistant strains.
Resistance to carbapenem in Pseudomonas aeruginosa and Acinetobacter baumannii is often due to loss of outer membrane proteins and upregulation of active efflux pumps or production of MBL. MBLs have been identified from clinical isolates worldwide with increasing frequency over the past few years and strains producing these enzymes have been responsible for prolonged nosocomial outbreaks that were accompanied by serious infections. The development of a simple and inexpensive screening method is necessary to detect the MBL production in microbiology laboratories. This is crucial for optimal treatment of patients particularly critically ill and hospitalized patients and to control the spread of resistance. Several studies have reported screening methods using metal chelators such as EDTA. We screened for MBL production by three methods and majority of the study isolates showed MBL production. Though molecular methods for the detection of MBL producing genes is the confirmatory test, easy and simple phenotypic tests are required for early identification. EDTA disk screening test is a useful tool for the clinical laboratories to notify the treating physicians and also devise methods to contain their spread.

In this study zone enhancement with imipenem–EDTA impregnated disc proved to be useful in screening for MBL. Though the gold standard among the phenotypic methods is the determination of MIC to carbapenem with and without EDTA combination, this cannot be routinely performed in all laboratories. Zone enhancement with EDTA impregnated antibiotic disc is simple, easy to perform and correlates well with the results of the MIC tests.

Routine disc susceptibility testing cannot always detect MBL. It indicates only resistance to carbapenems. This resistance can be mediated by multiple mechanisms. Detection of MBL is important because it calls for infection control measures to arrest the spread of MDR strains. The MIC to carbapenem may not be significantly raised in MBL-producing strains. Hence it is important to perform simple screening methods.

There was an overall mortality rate of 57% in these patients. Among the patients with Acinetobacter baumannii infection (n=25) 68% expired while 47% of patients with Pseudomonas aeruginosa (n=30) infection expired. A detailed clinical history was sought to rule out colonization and also various factors which influenced the mortality in the study patients.

The individual factors identified as contributing to mortality included stay in the ICU, mechanical ventilation and the usage of other invasive devices. These factors portray a severely ill patient who receives intensive nursing and for whom, the disease treatment and the invasive devices compromise the protective barriers. ICU stay had been found in previous studies to be an important risk factor for acquisition of resistant organisms and also more than half of the patients hospitalized in ICU acquire a nosocomial infection. Several studies have reported screening methods using metal chelators such as EDTA. We screened for MBL production by three methods and majority of the study isolates (n=44) showed MBL production. Though molecular methods for the detection of MBL producing genes is the confirmatory test, easy and simple phenotypic tests are required for early identification. EDTA disk screening test is a useful tool for the clinical laboratories to notify the treating physicians and also devise methods to contain their spread.

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**Table 1: Univariate analysis of factors influencing mortality in patients infected with multidrug resistant strains**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Recovered</th>
<th>Expired</th>
<th>Total</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean age±41.42 years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤40 yrs</td>
<td>11 (44%)</td>
<td>14 (56%)</td>
<td>25</td>
<td>0.960</td>
</tr>
<tr>
<td>&gt;40 yrs</td>
<td>13 (43.3%)</td>
<td>17 (56.7%)</td>
<td>30</td>
<td>0.739</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>18 (45%)</td>
<td>22 (55%)</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>6 (40%)</td>
<td>9 (60%)</td>
<td>15</td>
<td>0.001</td>
</tr>
<tr>
<td>Stay in ICU</td>
<td>17 (35.5%)</td>
<td>31 (64.6%)</td>
<td>48</td>
<td>0.001</td>
</tr>
<tr>
<td>Non ICU</td>
<td>7 (100%)</td>
<td>0</td>
<td>7</td>
<td>0.0005</td>
</tr>
<tr>
<td>Intubated</td>
<td>12 (28.6%)</td>
<td>30 (71.4%)</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Not intubated</td>
<td>12 (92.3%)</td>
<td>10 (7.7%)</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Presence of focal /generalized infection</td>
<td>9 (26.5%)</td>
<td>25 (73.5%)</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Absence of focal /generalized infection</td>
<td>15 (71.4%)</td>
<td>6 (28.6%)</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>8 (36.4%)</td>
<td>14 (63.6%)</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>16 (48.5%)</td>
<td>17 (51.5%)</td>
<td>33</td>
<td>0.375</td>
</tr>
<tr>
<td>Carbapenem used</td>
<td>8 (32%)</td>
<td>17 (68%)</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Carbapenem not used</td>
<td>16 (53.3%)</td>
<td>14 (46.7%)</td>
<td>30</td>
<td>0.112</td>
</tr>
<tr>
<td>Antibiotics administered</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta lactam</td>
<td>9 (60%)</td>
<td>6 (40%)</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Aminoglycosides/flouroquinolones</td>
<td>1 (100%)</td>
<td>0</td>
<td>1</td>
<td>0.144</td>
</tr>
<tr>
<td>Multiple combinations</td>
<td>14 (35.9%)</td>
<td>25 (64.1%)</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Indwelling devices</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial /urinary catheter</td>
<td>9 (37.5%)</td>
<td>2 (6.5%)</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Multiple devices</td>
<td>15 (62.5%)</td>
<td>29 (93.5%)</td>
<td>44</td>
<td>0.016</td>
</tr>
<tr>
<td>Surgical interventions</td>
<td>14 (45.2%)</td>
<td>17 (54.8%)</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>No surgical interventions</td>
<td>10 (41.7%)</td>
<td>14 (58.3%)</td>
<td>24</td>
<td>0.796</td>
</tr>
<tr>
<td>Hospital stay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean duration (24.4 days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤14 days</td>
<td>9 (40.9%)</td>
<td>13 (59.1%)</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>&gt;14 days</td>
<td>15 (45.5%)</td>
<td>18 (54.5%)</td>
<td>33</td>
<td>0.739</td>
</tr>
</tbody>
</table>

*P<0.05 is significant*
study patients. Presence of focal or generalized infection was a significant factor influencing the mortality in these patients. The eradication of the nidus of infection by debridement and other surgical procedures and removal of prosthetic devices has been reported to result in better patient outcomes.1,2

The intensity of selection pressure for usage of broad spectrum antibiotics is high in the ICU resulting in the eradication of competitive flora and selection of multidrug resistant strains.10,11 However in this study, eventhough multiple antibiotics were used in most of the study patients, this factor did not influence the mortality significantly.

Among the study patients14 those who had exposure to carbapenem prior to isolation of the resistant strain,11 patients continued to receive carbapenem even after the isolation of resistant strains, but they were put on combination therapy with aminoglycosides or quinolones. Thirty patients had no recorded evidence of exposure to carbapenems. Our centre being a tertiary care centre acquisition of resistant strains may have occurred in the referring hospitals where unrestricted use of carbapenems is known to occur or from this hospital after admission through horizontal transfer. This also implies that such strains may be imported by the patients into the hospital on admission and serve as potential source for dissemination. This calls for increased vigilance and enhanced infection control policies and practices.

No specific underlying disease was evidently associated with increased mortality rate. Performance of a surgical procedure was not statistically significant in influencing the mortality. This is supported by previous studies which assessed risk factor for acquiring pan-drug-resistant strains and their influence on the outcomes.10

Polymyxins B and colistin remain the mainstay of treatment for multi-drug resistant (MDR) Pseudomonas aeruginosa and Acinetobacter baumannii. The alternative therapeutic strategies for MDR Acinetobacter baumannii includes the use of rifampicin, tigecycline, minocycline or tigecycline with sulbactam.15 For MDR Pseudomonas aeruginosa inhibitors of multidrug efflux systems and new betalactamase inhibitors that are highly active against amp C and/or metalloenzymes are the subject of intensive investigations and could become valuable tools in the treatment of multidrug resistant strains.16

In this study eventhough a high proportion of the isolates (92%) were susceptible to colistin and polymyxin B, these drugs were not administered for treatment either alone or in combination. Though nephrotoxicity was a dreaded complication of colistin in the past, it is no longer considered a high risk drug and can be administered to patients with reasonably good renal functions, either alone or in combinations. And now evidence shows that polymyxins have less toxicity than previously reported.15 Since colistin was not administered to any of the patients in this study, the treatment outcomes could not be measured. The increased mortality observed in these patients may be in part due to deferment of colistin. The attributable mortality due to carbapenem resistance could not be ascertained since the study was not a cohort study.

Susceptibility testing for antimicrobials that are not tested routinely and in these antibiotic synergy studies should be considered.7 Furthermore, combination therapy may keep a selection pressure that allows only subpopulations with reduced virulence to be expressed. Modes of delivery that achieve high drug concentration at the infection site may be tried. Aggressive surgical interventions to remove the nidus of infection should be sought.1,2 Aboveall strict infection control precautions should be undertaken to contain the strains from reaching epidemic proportions.

Acquisition of multidrug resistant Acinetobacter baumannii and Pseudomonas aeruginosa are related to environmental contamination and to contact with transiently colonised healthcare providers. Control measures addressing these sources of infection have been proved to be successful in controlling the spread of these organisms. Hence continued careful attention to hand hygiene, contact isolation, barrier precautions, adequate environmental cleaning and careful disinfection of patient care equipment along with surveillance are essential to prevent outbreak of infections caused by multidrug resistant strains.18

Conclusion

Resistance to carbapenem by Pseudomonas aeruginosa and Acinetobacter baumannii is a factor with significant threat in hospitals. This should be addressed with alternative and newer therapeutic strategies, strict infection control measures and continued surveillance.

References

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17. Falagas ME and Kasiakou SK. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. Critical Care 2006, 10:R27


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**Announcement**

**ISHTMGOLDCON 2009**

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**Announcement**

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